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Research Article

Assessment of rice (Co 51) seed ageing through volatile organic compound analysis using Headspace-Solid Phase Micro Extraction/ Gas Chromatography-Mass Spectrometry (HS-SPME/GCMS)

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Abstract
Seed ageing is an inevitable process that reduces seed quality during storage. When seeds deteriorate as a result of the lipid peroxidation process, it leads to produce toxic volatile organic compounds. These volatiles served as an indicator for the viability of stored seeds. With this background, the study was conducted to profile the volatile organic compounds emitted from rice seeds during storage. Volatile profiling of stored rice var. Co 51 seeds was done through Headspace-Solid phase microextraction/ Gas chromatography-mass spectrometry (HS-SPME/GCMS). The study clearly demonstrated that the significant decrease in physiological and biochemical quality attributes was noted due to an increase in the strength of volatiles released during ageing. When the release of total volatile strength reached more than 40%, a significant reduction in physiological attributes such as germination, root and shoot length, dry matter production and vigour index were observed. With respect to biochemical properties, a significant increase in electrical conductivity of seed leachate, lipid peroxidation and lipoxygenase activity, and decrease in dehydrogenase, catalase and peroxidase activities were observed. However, the highest reduction in all these properties were recorded when the total volatile strength reached to 54.90%. Finally, the study concluded that, among all the volatiles, 1-hexanol, 1-butanol, ethanol, hexanal, acetic acid, hexanoic acid and methyl ester were the most closely associated volatiles with seed deterioration. It indicates that these components could be considered the signature components for assessing the seed quality in rice during storage.

Keywords: Rice, Seed deterioration, Seed quality, Volatile organic compounds

INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple food crop for billions of people and is grown in over fifty nations across the world. It belongs to the family poaceae. India has the largest area of rice cultivation after China

and is the major consumer in the world. In India, rice is cultivated in an area of 43.66 million ha, producing 188.37 million tonnes with a productivity of 2722 kg/ha. Tamil Nadu accounts for a rice area of 1.90 million ha, production of 7.71 million tonnes and productivity of 3760 kg/ha (Indiastat, 2020).

During storage, major losses of seeds are caused by various biological and non-biological factors, including fungi, temperature, humidity and seed moisture level which, play a major role in the deterioration of seeds, including seed rots, molding of seeds, pre and post-emergence damping-off, low seed viability and poor seedling growth. Hence, there is a need to examine reasonable factors of these storage losses, which ultimately affect the market value and quality of the seeds (Mira *et al*., 2016; Bhattacharjee., 2019).

Stored seeds produce increased level of volatile organic compounds that leads to seed deterioration. Many volatile organic compounds (VOCs) are reactive and toxic, perpetuating reactions that lead to deterioration and accelerating the rate at which seeds lose viability for high concentrations of ethanol and acetaldehyde (Akimoto *et al*., 2004). VOCs are the major by-product of catabolic reactions and volatile alka(e)nes and aldehydes are major by-products of lipid peroxidation (Grotto *et al*., 2009). Saturated aldehydes such as hexanal are the potential biomarkers of lipid peroxidation during storage of seeds. These volatile organic compounds are toxic to seeds and give a path to free radical production and finally deterioration of seeds during storage (Meenakshi, 2020; Umarani *et al.,* 2020).

Hence, the present study was conducted to profile the volatile organic compounds emitted from rice seeds during storage and identify the major volatile organic compounds involved in seed deterioration.

MATERIALS AND METHODS

Genetically pure seed lots of Rice var. Co 51 obtained from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore were used as the base seed materials for this study. The storage experiment was carried out at the Department of Seed Science and Technology and Gas Chromatography Mass Spectrometry (GC-MS) analysis of volatile organic compounds was carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

Rice seeds were stored in air-tight containers (screw-capped glass bottles) of 500g capacity. The cap was provided with a septum to facilitate the air sampling from the glass bottle. The glass bottles were filled with 100g of seeds. The glass bottles were kept under ambient conditions for a storage period of 12 months.

Profiling of volatile organic compounds through HS-SPME/GC-MS

Sampling and extraction of volatile organic compounds

The air sample was taken monthly from the glass bottle using solid-phase micro extraction (SPME) fiber. While taking the sample the SPME fiber was inserted just nearing the surface of the seed layer. Adsorption time for collection of air sample using SPME fiber was 30 min. Then the collected air sample of SPME fiber was directly injected to GC-MS (Thermo Scientific Trace GC Ultra chromatograph system, coupled to

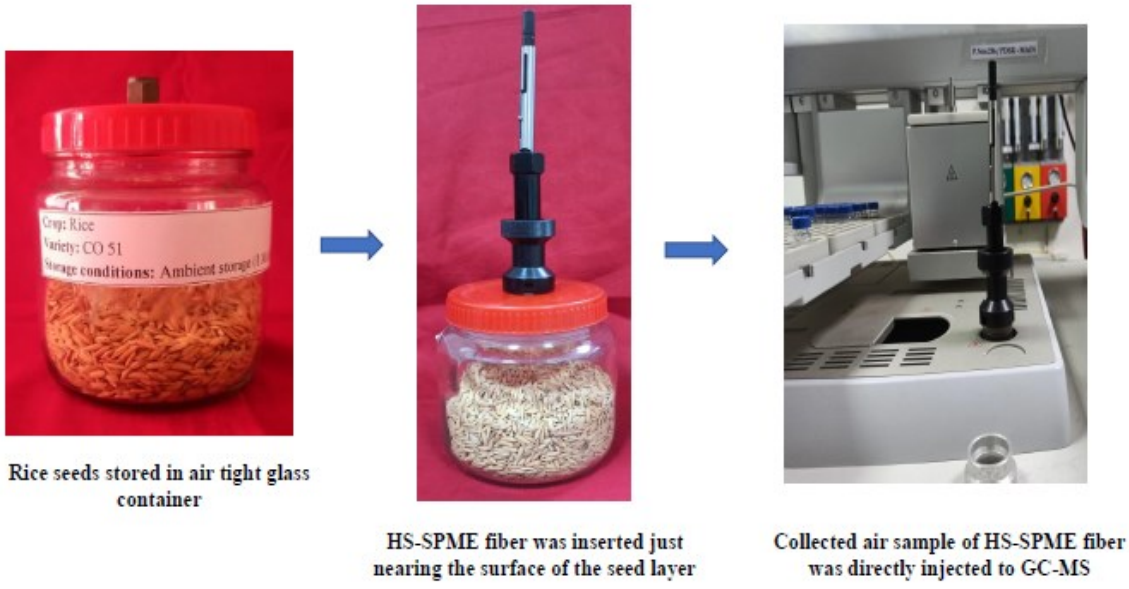

Plate 1. *VOCs analysis of rice seeds using HS-SPME/GC-MS*

thermo scientific DSQ II quadruple mass spectrometer) (Plate 1). The Helium (99.9%) gas was used as a carrier gas with flow rate of 1.0 ml/min and pressure of (60-100 Psi, 400-700 Kpa). Volatile compounds from air sample were separated by phenyl methyl silicon fused-silica capillary column (TG-5 MS, 30m in length, 0.25µm and 0.25µm film thicknesses).

Volatiles were extracted and concentrated using SPME manual holder assembly equipped with SPME fiber conditioned at 250ºC for 30 min. The fiber was desorbed at 250ºC injector temperature in splitless mode. The GC oven was programmed as 1 min hold at 50ºC, ramped to 100ºC at the rate of 4ºC/min and was further ramped to 240ºC at the rate of 50ºC /min with final hold of 2 min. Injection volume of 1µl was taken in split less mode. The injector and detector were constantly maintained at temperature of 250ºC and 260ºC, respectively, with a total run time of 1hr for good separation of the diverse compounds. Then the volatile organic compounds were identified by the fragmentation pattern of individual compounds and confirmed with the NIST (National Institute of Standards and Technology) Library database (Mathure *et al.,* 2011).

Evaluation of physiological and biochemical seed quality attributes

The rice seeds were tested for different physiological seed quality parameters such as germination percentage, shoot and root length, dry matter production and vigour index as per ISTA seed testing protocols (ISTA, 2019) and the biochemical seed quality parameters such as electrical conductivity of the seed leachate (Presley, 1958), dehydrogenase enzyme activity (Kittock and law, 1968), catalase activity (Aebi, 1984), peroxidase activity (Malik and Singh, 1980), lipid peroxidation activity (Bernheim *et al*., 1948) and lipoxygenase activity (Hildebrand *et al*., 1993).

Statistical analysis

Data obtained from the experiments were analysed using an analysis of variance (ANOVA) as a factorial combination of treatments. Mean values were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at 0.05 probability level. Values in per cent data were arcsine transformed before analysis.

RESULTS AND DISCUSSION

Profiling of volatile organic compounds

Volatile organic compounds profiling of stored rice seeds was done through GC-MS. The volatiles were analysed at monthly interval. The results showed that several volatile components were released from stored rice seeds and all the components fall into eight major groups such as alcohols, aldehydes, acids, esters, alkanes, alkenes, ketones and ethers.

In the alcohol group, 1-butanol was emitted from 2nd month of storage to continue till the end of storage period. 1-hexanol was emitted from 1st month of storage to 6 months after storage. Ethanol, 1-monolinolenyl glycerol and 2-hexadecanol were emitted from 9 month after storage and continued till the end of the storage period.

Among the volatile alcohol compounds, 1-butanol and 1-hexanol were the most abundant volatiles, and the area percentage was progressively increased during the storage period. The area percentage of 1-butanol at initial was 0.49 per cent and increased to 8 months after storage (5.62 per cent) followed by 1-hexanol, which started from 0.93 per cent and increased up to 4 months after storage (5.73 per cent), thereafter decreased. Even though some of the other alcohol compounds were released during the storage period, their area percentage was low for the whole period of storage (Table 1). The most abundant release of 1-hexanol and 1-butanol might be due to the anaerobic metabolism or from glycolysis pathway also involved due to lipid peroxidation. This may also happen because of the loss in mitochondrial membrane integrity (Colville *et al*., 2012). Buckley and Buckley (2009) reported the emission of ethanol and 1-butanol in canola seeds during ageing due to anaerobic metabolism. According to Meenakshi (2020), ethanol and 1-hexanol was emittedd in very slow rate and detected in dry stored seeds of sunflower and they stated that the glycolytic reactions in stored seeds produce the ethanol and other alcoholic groups.

In the aldehyde group, hexanal was emitted from 1st month of storage to continue till the end of the storage period. Octadecanal was released 7 month after storage and continued till the end of the storage period. Among aldehydes, hexanal was observed to have a higher area percentage. Its contribution was substantially increased from 2.57 per cent at 1st month to 5.28 per cent in 3rd month followed by Octadecanal, which was increased from 0.48 per cent at 7th month to 2.83 per cent at the end of storage period (Table 1). The release of hexanal from aged seeds might be due to the disintegration of linoleic polyunsaturated fatty acids by auto-oxidation or by enzymatic oxidation and also attributed to mitochondrial damage. Aldehydes are the major volatiles emitted due to lipid peroxidation, hexanal, Nonenal and 2,4-Nonadienal were the volatiles associated with the degradation of linoleic acid (Meenakshi *et al.,* 2020). According to Umarani *et al.,* (2020) the changes in the release of volatile aldehydes, especially malondialdehyde due to lipid peroxidation process found to highly reduced the seed viability. The second oxidative stress messenger namely 4-hydroxy-2,3-

Table 1. Area percentage (strength) of volatile organic compounds (VOCs) emitted from rice seeds during storage

Compounds	Period of storage (Months)											
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}
Alcohols (13)												
1-Hexanol	0.93	1.78	3.05	5.73	3.16	0.72	0	0	0	0	0	0
1-Butanol	0	0.49	0.94	1.29	1.57	2.33	3.84	5.62	3.04	1.82	1.38	1.02
2-Propyl-1-pentanol	0.21	0.63	0	0	0	0	0	0	0	0	0	0
4-Methyl-2-hexanol	0	0	0.14	0.83	0	0	0	0	0	0	0	0
5-Methyl-2-hexanol	0	0	0	0	0.71	0	0	0	0	0	0	0
1-Phenoxy-2-propanol	0	0	0	0	0.28	0.49	0	0	0	0	0	0
1-Pentanol	0	0	0	0	0.13	0.37	0	0	0	0	0	0
Hexaethylene glycol	0	0	0	0	0.32	0.55	0	0	0	0	0	0
Heptaethylene glycol	0	0	0	0	0	0.18	0.27	0	0	0	0	0
Octaethylene glycol	0	0	0	0	0	0.13	0.35	0.71	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0.93	1.13	1.72	3.07
1-Monolinolenyl glycerol	0	0	0	0	0	0	0	0	0.72	0.87	1.05	1.93
2-Hexadecanol	0	0	0	0	0	0	0	0	1.02	4.92	6.02	8.26
Total	1.14	2.90	4.13	7.85	6.17	4.77	4.46	6.33	5.71	8.74	10.17	14.28
Aldehydes (8)												
Hexanal	2.57	2.93	5.28	2.08	1.81	0.95	0.63	0.44	0.37	0.31	0.19	0.12
Benzaldehyde	0	0	0	0.73	0.87	1.03	1.35	1.51	0	0	0	0
2,6-Dimethyl benzaldehyde	0	0.38	0.62	0	0	0	0	0	0	0	0	0
2-Methylbutanal	0	0.32	0	0	0	0	0	0	0	0	0	0
3-Methylbutanal	0	0	0.18	0.49	0	0	0	0	0	0	0	0
Nonanal	0.13	0.73	0	0	0	0	0	0	0	0	0	0
Octadecanal	0	0	0	0	0	0	0.48	0.72	0.91	1.17	1.33	2.83
10-Octadecenal	0	0	0	0	0	0	0	0	0	0	0.72	1.07
Total	2.70	4.36	6.08	3.30	2.68	1.98	2.46	2.67	1.28	1.48	2.24	4.02
Acids (15)												
Arsenous acid	0.32	0.72	0.98	0	0	0	0	0	0	0	0	0
4-Ethyl benzoic acid	0	0	0	0	0	0	0.59	0.72	0	0	0	0
Benzoic acid	0.83	0.97	1.14	1.63	1.69	1.93	2.49	2.71	2.83	3.02	0	0
2-Trimethylsiloxy-6-hexadecenoic acid	1.02	0	0	0	0	0	0	0	0	0	0	0
Stearic acid	0.89	1.03	1.47	0	0	0	0	0	0	0	0	0
Butanoic acid	0	0.44	0.72	0.91	1.17	1.42	1.77	1.92	2.33	2.51	2.75	0.82
Octadecanoic acid	0.07	0.73	0	0	0	0	0	0	0	0	0	0
Acetic acid	0	0.74	1.12	1.92	2.28	2.73	3.35	3.61	3.91	4.11	4.82	3.02
Propanoic acid	0	0	0	0	0	0	0.35	0.52	0.77	0.93	1.19	1.33
Pentanoic acid	0	0	0	0	0	0	0	0.46	0.72	0	0	0
Hexanoic acid	0	0	0.24	0.58	3.94	5.03	5.97	3.02	1.96	1.18	1.03	0.45
Docosanoic acid	0	0	0	0	0	0	0.62	0.98	1.12	0	0	0
Decanoic acid	0	0	0	0	0	0	0.34	0.83	1.08	1.28	1.55	1.72
Hexadecanoic acid	0	0	0	0	0	0	0	0	0.73	0.99	1.13	1.48
Tetradecanoic acid	0	0	0	0	0	0	0	0	0.28	0.33	0.72	1.06
Total	3.13	4.63	5.67	5.04	9.08	11.11	15.48	14.77	15.73	14.35	13.19	9.88

Contd…….

Table 1. Contd…….

Esters (18)												
Methyl ester	1.28	1.73	1.97	2.08	2.49	2.72	2.93	2.98	3.14	3.34	3.72	4.02
Ethyl ester	0	0	0.72	0.77	1.02	1.09	0	0	0	0	0	0
Phenyl methyl ester	1.05	0	0	0	0	0	0	0	0	0	0	0
3-Propyl ester	0.82	0	0	0	0	0	0	0	0	0	0	0
3-Mercapto hexyl ester	0.39	0	0	0	0	0	0	0	0	0	0	0
6-Ethyl-3-octyl ester	0.44	0	0	0	0	0	0	0	0	0	0	0
2-Ethyl ester	0	0.48	0	0	0	0	0	0	0	0	0	0
Propyl ester	0	0.64	0.82	1.03	1.38	1.62	1.68	1.92	2.04	2.17	2.49	3.11
Eicosyl ester	0	0.62	0.93	0	0	0	0	0	0	0	0	0
Trimethyl silyl ester	0.77	0.97	1.39	1.69	0	0	0	0	0	0	0	0
Octyl ester	0	0	0	0	0	0	0.31	0.35	0.48	0.62	0	0
Octa decyl ester	0	0	0	0	0	0	0.18	0	0	0	0	0
Butyl tetradecyl ester	0	0	0	0	0	0	0.72	0.88	0	0	0	0
2-Butyl ester	0	0	0	0	0	0	0.12	0.17	0.22	0.37	0.58	0.77
1,2,3-Propanetriyl ester	0	0	0	0	0	0	0.52	0.72	0.91	1.18	0	0
Diethyl ester	0	0	0	0	0	0	0	0	0	0.63	0.81	1.16
9,9a-Didecenoate	0	0	0	0	0	0	0	0	0	0.14	0.27	0.44
Cyclohexyl ester	0	0	0	0	0	0	0	0	0	0	1.04	1.38
Total	4.75	4.44	5.83	5.57	4.89	5.43	6.46	7.02	6.79	8.45	8.91	10.88
Alkanes (16)												
Butane	1.17	1.42	1.68	1.95	2.06	2.38	0	0	0	0	0	0
Tetradecane	0	0.43	0	0	0	0	0	0	0	0	0	0
Undecane	0	0	0.97	1.38	1.72	1.88	0	0	0	0	0	0
Heptane	0	0	0.18	0.42	0	0	0	0	0	0	0	0
Pentane	0	0	0	0.93	0	0	0	0	0	0	0	0
1,1-Dichloropentane	0	0	0	0.18	0.37	0	0	0	0	0	0	0
3-Ethyl-3-methylheptane	0	0	0	0.36	0.73	0	0	0	0	0	0	0
5,5-Diethyl heptade-cane	0	0	0	0.05	0.28	0	0	0	0	0	0	0
Hexadecane	0	0	0	0.22	0.58	0.82	0.93	3.98	7.02	7.97	4.18	0
Dodecane	0	0	0	0	0.04	0.23	0	0	0	0	0	0
Octane	0	0	0	0	0	0	0	0.61	0.88	0	0	0
Octadecane	0	0	0	0	0	0	0.52	0.73	0.92	1.02	1.55	1.93
Tetratetracontane	0	0	0	0	0	0	0.17	0.28	0.48	0.66	0.83	1.02
1,2-Epoxynonane	0	0	0	0	0	0	0.22	0	0	0	0	0
17-Pentatriacontane	0	0	0	0	0	0	0	0	0.72	0.79	0.92	1.29
3,3,3-Hexafluropropane	0	0	0	0	0	0	0	0	0	0	0.64	1.19
Total	1.17	1.85	2.83	5.49	5.78	5.31	1.84	5.60	10.02	10.44	8.12	5.43
Alkenes (5)												
Cyclobutene	0	0	0	0.04	0	0	0	0	0	0	0	0
Benzene	0	0	0	0.27	0.48	0.59	0.83	0	0	0	0	0
Humulene	0	0	0.17	0.38	0.77	0.91	0	0	0	0	0	0
à-Pinene	0	0	0	0	0	0	0	0	0	0	0.36	0.78
à-Phellandrene	0	0	0	0	0	0	0	0	0	0	0	0.26
Total	0	0	0.17	0.69	1.25	1.50	0.83	0	0	0	0.36	1.04
Ketones (4)												
2-Heptanone	0	0	0.37	0.55	0.82	0.96	0	0	0	0	0	0
2,6-Dihydroxy ace-tophenone	0	0	0	0	0	0	0.28	0.55	0.71	1.05	1.47	3.82
Methanethione	0	0	0	0	0	0	0.13	0.27	0	0	0	0
2-Nonadecanone	0	0	0	0	0	0	0	0	0.38	0.57	0.86	1.45
Total	0	0	0.37	0.55	0.82	0.96	0.41	0.82	1.09	1.62	2.33	5.27
Ethers (2)												
Trimethyl silyl ether	0	0	0	0	0.13	0.33	0.56	0.81	0.97	1.18	1.62	2.28
Monododecyl ether	0	0	0	0	0	0	0	0	0	0.27	0.56	1.82
Total	0	0	0	0	0.13	0.33	0.56	0.81	0.97	1.45	2.18	4.10
Grand Total	12.89	18.18	25.08	28.49	30.80	31.39	32.50	38.02	41.59	46.53	47.50	54.90

nonenal (HNE) is another widely researched most cytotoxic aldehyde that reacts easily with DNA, nucleic acids, proteins and phospholipids, thereby influencing gene expression and causing damage to viable tissues of seeds.

In the acid group, acetic acid, hexanoic acid and butanoic acid were released almost throughout the storage period. Benzoic acid was emitted from 1st month of aged seeds and thereafter, they continued to release up to 10 months of storage. Acetic acid, hexanoic acid and butanoic acid were the dominant acids and their concentrations increased with storage time. Initially, the percentage contribution of acetic acid, hexanoic acid and butanoic acid were 0.74, 0.24 and 0.44 per cent,

Table 2. Effect of VOCs emission levels on physiological quality of rice seeds during storage

Period of storage (Months)	Germination (%)	Root length (cm)	Shoot length (cm)	VOCs strength (%)		
				Strength of individual VOC group	Dominant individual VOC	Total VOCs strength
P_1	91 (72.54)	21.5	10.5	Alcohol (1.14), Aldehyde (2.70), Acids (3.13), Esters (4.75), Alkanes (1.17), Alkenes (0), Ketones (0), Ethers (0)	Hexanal (2.57) Methyl ester (1.28)	12.89
P_2	89 (70.63)	21.6	10.4	Alcohol (2.90), Aldehyde (4.36), Acids (4.63), Esters (4.44), Alkanes (1.85), Alkenes (0), Ketones (0), Ethers (0)	Hexanal (2.93) 1-Hexanol (1.78)	18.18
P_3	88 (69.73)	20.8	10.1	Alcohol (4.13), Aldehyde (6.08), Acids (5.67), Esters (5.83), Alkanes (2.83), Alkenes (0.17), Ketones (0.37), Ethers (0)	Hexanal (5.28) 1-Hexanol (3.05)	25.08
P_4	88 (69.73)	20.4	9.6	Alcohol (7.85), Aldehyde (3.30), Acids (5.04), Esters (5.57), Alkanes (5.49), Alkenes (0.69), Ketones (0.55), Ethers (0)	1-Hexanol (5.73) Hexanal (2.08)	28.49
P_5	86 (68.03)	18.9	9.6	Alcohol (6.17), Aldehyde (2.68), Acids (9.08), Esters (4.89), Alkanes (5.78), Alkenes (1.29), Ketones (0.82), Ethers (0.13)	Hexanoic acid (3.94) 1-Hexanol (3.16)	30.80
P_6	84 (66.42)	19.1	9.3	Alcohol (4.77), Aldehyde (1.98), Acids (11.11), Esters (5.43), Alkanes (5.31), Alkenes (1.50), Ketones (0.96), Ethers (0.33)	Hexanoic acid (5.03) Acetic acid (2.73)	31.39
P_7	83 (65.65)	18.5	9.4	Alcohol (4.46), Aldehyde (2.46), Acids (15.48), Esters (6.46), Alkanes (1.84), Alkenes (0.83), Ketones (0.41), Ethers (0.56)	Hexanoic acid (5.97) 1-Butanol (3.84)	32.50
P_8	81 (64.15)	18.5	9.1	Alcohol (6.33), Aldehyde (2.67), Acids (14.77), Esters (7.02), Alkanes (5.60), Alkenes (0), Ketones (0.82), Ethers (0.81)	1-Butanol (5.62) Acetic acid (3.61)	38.02
P_9	80 (63.44)	18.2	9.3	Alcohol (5.71), Aldehyde (1.28), Acids (15.73), Esters (6.79), Alkanes (10.02), Alkenes (0), Ketones (1.09), Ethers (0.97)	Hexadecane (7.02) 1-Butanol (3.04)	41.59
P_{10}	77 (61.34)	18.4	8.8	Alcohol (8.74), Aldehyde (1.48), Acids (14.35), Esters (8.45), Alkanes (10.44), Alkenes (0), Ketones (1.62), Ethers (1.45)	Hexadecane (7.97) 2-Hexadecanol (4.92)	46.53
P_{11}	74 (59.34)	17.5	8.5	Alcohol (10.17), Aldehyde (2.24), Acids (13.19), Esters (8.91), Alkanes (8.12), Alkenes (0.36), Ketones (2.33), Ethers (2.18)	2-Hexadecanol (6.02) Acetic acid (4.82)	47.50
P_{12}	68 (55.55)	17.2	8.6	Alcohol (14.28), Aldehyde (4.02), Acids (9.88), Esters (10.88), Alkanes (5.43), Alkenes (1.04), Ketones (5.27), Ethers (4.10)	2-Hexadecanol (8.26) Hexadecane (4.02)	54.90
Mean	82(64.90)	19.22	9.43			
SEd	1.66	0.39	0.17			
CD (P=0.05)	3.42	0.80	0.36			

(Figure in parenthesis indicate arcsine values)

respectively. Afterwards, it increased with the advancement in storage time. They accounted for about 4.82, 5.97 and 2.75 per cent, respectively. Even though some of the other acid compounds were released during the storage period, their area percentage was low for the whole period of storage (Table 1).

In the ester group, methyl ester and propyl ester was released throughout the storage period. Initially the area percentage contribution of methyl ester and propyl ester was 1.28 and 0.64 per cent, respectively; afterwards, it increased at the end of the storage period 4.02 and 3.11 per cent, respectively (Table 1).

In the alkane group, butane was found to be released in the 1st month of aged seeds and thereafter continued to release up to 6 months after storage. Octadecane and tetratetracontane appeared in 7 month of aged seeds; thereafter, they continued to release until the end of the storage period. All the alkane com-

Table 3. Effect of VOCs emission levels on dry matter production and vigour index of rice seeds during storage

Period of storage (Months)	Dry matter production (g 10 seedlings^{-1})	Vigour index	VOCs strength (%)		
			Strength of individual VOC group	Dominant individual VOC	Total VOCs strength
P_1	0.077	2912	Alcohol (1.14), Aldehyde (2.70), Acids (3.13), Esters (4.75), Alkanes (1.17), Alkenes (0), Ketones (0), Ethers (0)	Hexanal (2.57) Methyl ester (1.28)	12.89
P_2	0.071	2848	Alcohol (2.90), Aldehyde (4.36), Acids (4.63), Esters (4.44), Alkanes (1.85), Alkenes (0), Ketones (0), Ethers (0)	Hexanal (2.93) 1-Hexanol (1.78)	18.18
P_3	0.068	2719	Alcohol (4.13), Aldehyde (6.08), Acids (5.67), Esters (5.83), Alkanes (2.83), Alkenes (0.17), Ketones (0.37), Ethers (0)	Hexanal (5.28) 1-Hexanol (3.05)	25.08
P_4	0.068	2640	Alcohol (7.85), Aldehyde (3.30), Acids (5.04), Esters (5.57), Alkanes (5.49), Alkenes (0.69), Ketones (0.55), Ethers (0)	1-Hexanol (5.73) Hexanal (2.08)	28.49
P_5	0.069	2451	Alcohol (6.17), Aldehyde (2.68), Acids (9.08), Esters (4.89), Alkanes (5.78), Alkenes (1.29), Ketones (0.82), Ethers (0.13)	Hexanoic acid (3.94) 1-Hexanol (3.16)	30.80
P_6	0.067	2386	Alcohol (4.77), Aldehyde (1.98), Acids (11.11), Esters (5.43), Alkanes (5.31), Alkenes (1.50), Ketones (0.96), Ethers (0.33)	Hexanoic acid (5.03) Acetic acid (2.73)	31.39
P_7	0.063	2316	Alcohol (4.46), Aldehyde (2.46), Acids (15.48), Esters (6.46), Alkanes (1.84), Alkenes (0.83), Ketones (0.41), Ethers (0.56)	Hexanoic acid (5.97) 1-Butanol (3.84)	32.50
P_8	0.062	2236	Alcohol (6.33), Aldehyde (2.67), Acids (14.77), Esters (7.02), Alkanes (5.60), Alkenes (0), Ketones (0.82), Ethers (0.81)	1-Butanol (5.62) Acetic acid (3.61)	38.02
P_9	0.058	2200	Alcohol (5.71), Aldehyde (1.28), Acids (15.73), Esters (6.79), Alkanes (10.02), Alkenes (0), Ketones (1.09), Ethers (0.97)	Hexadecane (7.02) 1-Butanol (3.04)	41.59
P_{10}	0.061	2094	Alcohol (8.74), Aldehyde (1.48), Acids (14.35), Esters (8.45), Alkanes (10.44), Alkenes (0), Ketones (1.62), Ethers (1.45)	Hexadecane (7.97) 2-Hexadecanol (4.92)	46.53
P_{11}	0.059	1924	Alcohol (10.17), Aldehyde (2.24), Acids (13.19), Esters (8.91), Alkanes (8.12), Alkenes (0.36), Ketones (2.33), Ethers (2.18)	2-Hexadecanol (6.02) Acetic acid (4.82)	47.50
P_{12}	0.055	1754	Alcohol (14.28), Aldehyde (4.02), Acids (9.88), Esters (10.88), Alkanes (5.43), Alkenes (1.04), Ketones (5.27), Ethers (4.10)	2-Hexadecanol (8.26) Hexadecane (4.02)	54.90
Mean	0.065	2373			
SEd	0.0012	50.09			
CD (P=0.05)	0.0025	103.40			

pounds area percentage was very low during the storage period. All 5 alkene compounds recorded very minimum area per cent in the alkene group. 2-heptanone, methanethione, 2,6-dihydroxy acetophenone and 2-nonadecanone belong to ketone group and trimethylsilyl ether and monododecyl ether belong to ether group were released during the storage period, but their area percentage was very low during the whole period of storage (Table 1).

The release of these volatile compounds during storage might be attributed to oxidation of lipid bi-layer cell membrane and also non-enzymatic degradation of macromolecules. Various chemical reactions such as glycolysis, auto-oxidation or non-enzymatic oxidation and strecker degradation of maillard reaction in aged seeds produce acids, alkanes, alkenes, esters, ketones and ethers (Mira *et al*., 2016). Lipid oxidation in stored pea seeds emitted a range of volatile organic compounds *viz*., ketones, alcohols, alkanes and esters (Bhattacharjee., 2019; Umarani *et al.,* 2020) and they also stated that these compounds are likely source for alcohols *viz*., pentanol, butanol and propanol, and also for *n*-hexylformate and 2-butanone.

VOCs emission levels and physiological seed quality during storage

In the present study, the results demonstrated the effect of VOCs emission level on physiological seed quality attributes. The germination, root and shoot length, dry matter production and vigour index were significantly reduced during storage corresponding with the increase in release of volatile organic compounds. The germination decreased from 91 per cent at 1st month to 68 per cent in 12th month of ageing. Initially (up to 4 months), there was no much reduction in germination during that time VOCs release strength was 28.49%. When VOCs strength increased beyond 40%, significant decrease in germination was noted in 9th month. At the end of storage period, the germination was 68 per cent, at the time VOCs release strength reached 54.90% and the individual VOCs released in higher strength are Alcohol (14.28%), Aldehyde (4.02%), Acids (9.88%), Esters (10.88%), Alkanes (5.43%), Alkenes (1.04%), Ketones (5.27%) and Ethers (4.10%). Likewise, the root length decreased from 21.5 cm in 1st month to 17.2 cm at 12th month of storage. Similarly, a significant reduction in shoot length from 10.5 cm at 1st month to 8.6 cm at 12th month was also recorded. The dry matter production was decreased from 0.077 g 10 seedling^{-1} at 1st month to 0.055 g 10 seedling^{-1} in 12th month and the vigour index also significantly reduced from 2912 at 1st month to 1754 in 12th month of ageing (Table 2, 3 & Fig. 1).

The deterioration in physiological parameters might be due to the toxic volatiles emitted from lipid oxidation of lipid bi-layer membrane and fermentation, which decline the mitochondrial activity resulting in reduced germination and seedling vigour. Balesevic *et al.* (2005) stated that the production of toxic volatiles like aldehydes, alcohols and ketones reduced the germination, seedling length, dry matter production and seedling vigour in sunflower. Volatile organic compounds, especially the aldehydes emanated from stored dry seeds, have reduced the germination and seedling vigour of pea and soybean seeds (Harman *et al.*, 1982). According to Woodstock and Taylorson (1981), soybean seeds' germination and seedling vigour were reduced due to the deleterious effect of toxic volatiles (acetaldehyde and ethanol) evolved by seeds during ageing and these compounds knockout the mitochondria and reduces the seed quality. Zhang *et al.* (1994) observed that acetaldehyde, acetic acid, ethanol, ethyl

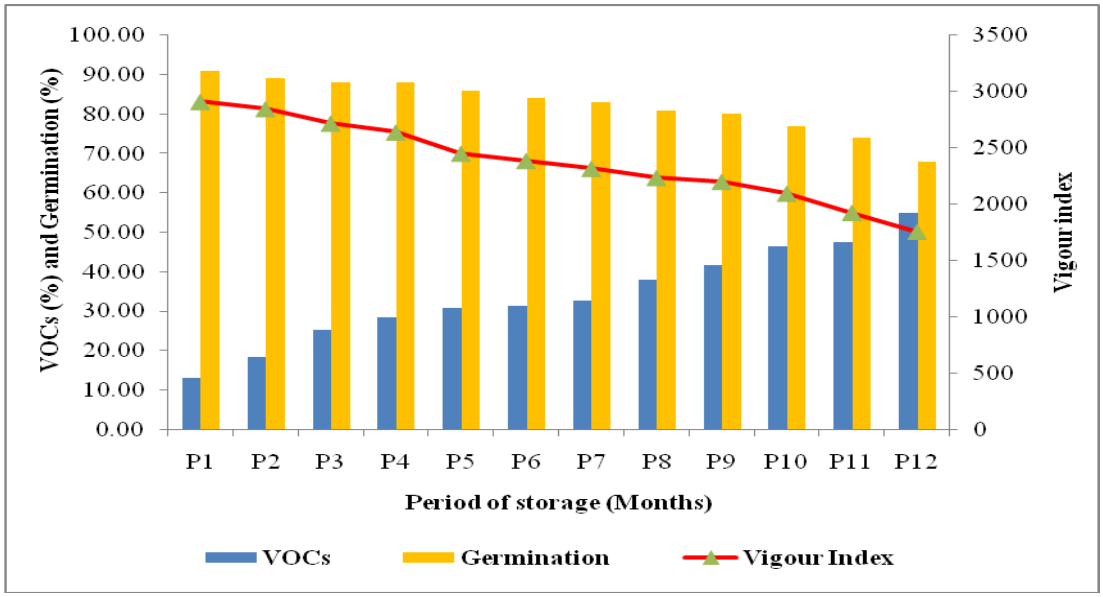

Fig. 1. *VOCs emission levels on germination percentage and vigour index of rice seeds during storage*

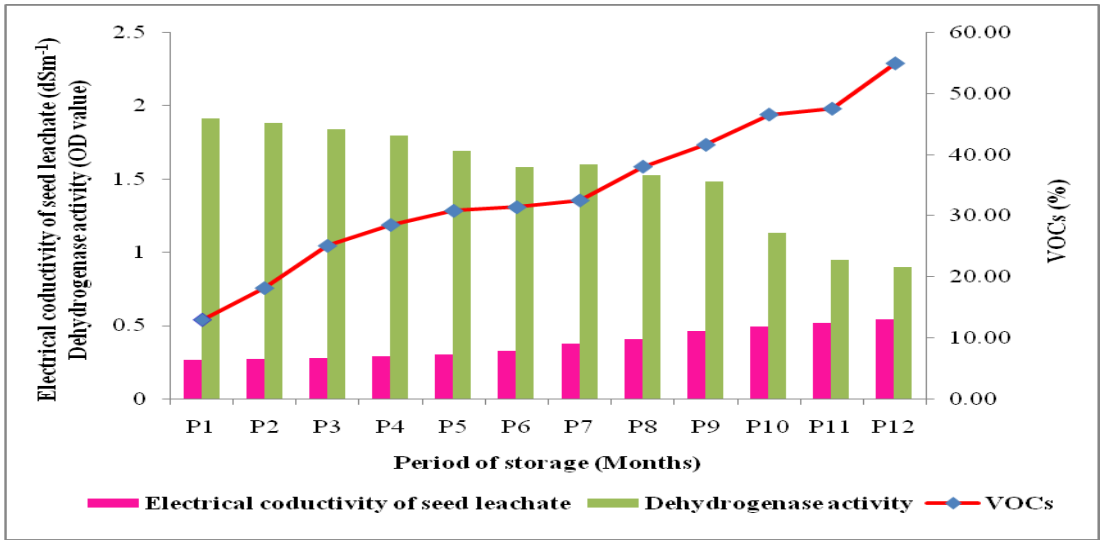

Fig. 2. *VOCs emission levels on electrical conductivity of seed leachate and dehydrogenase activity of rice seeds during storage*

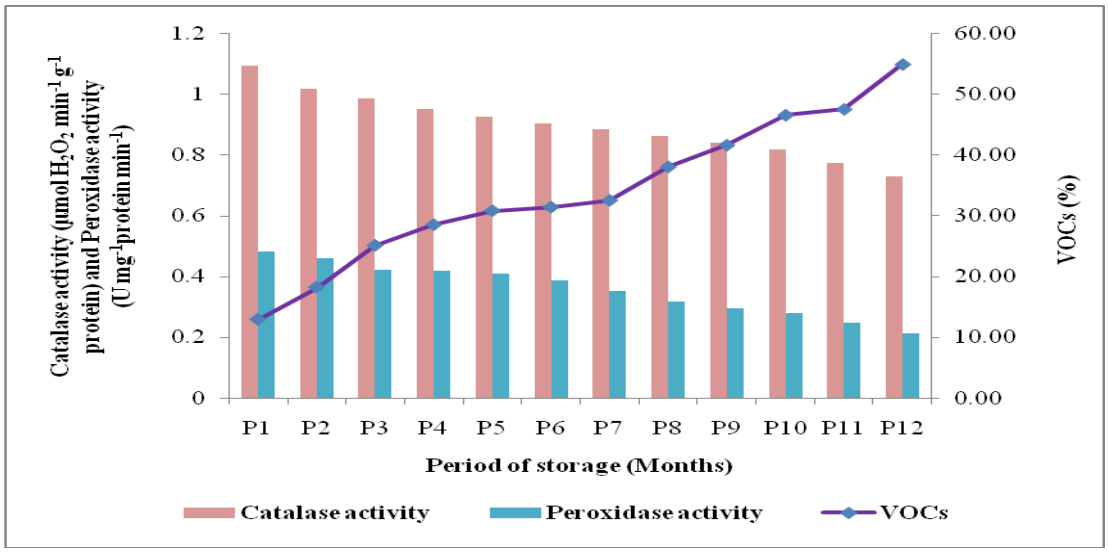

Fig. 3. *VOCs emission levels on catalase and peroxidase enzyme activity of rice seeds during storage*

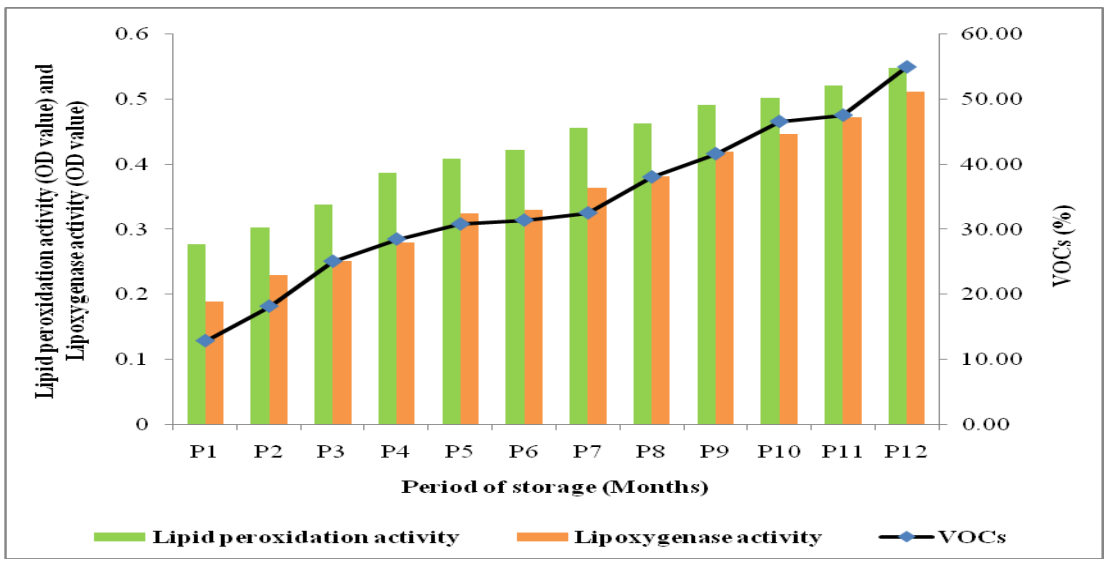

Fig. 4. *VOCs emission levels on lipid peroxidation and lipoxygenase activity of rice seeds during storage*

acetate, acetone and isopropanol evolved from seeds of rice and lettuce found to decline the germination and vigour regardless of storage environment and all the volatile organic compounds were negatively correlated with seed germination and seedling vigour tested over a period of storage. According to Umarani *et al.* (2020) regarding volatile emission from stored seeds, described the release of volatile organic compounds, *viz.,* hexanal, pentane, acetaldehyde and ethanol and their relation with seed quality deterioration and the use of volatile organic compounds as biomarkers for detecting the level of deterioration processes in seeds.

VOCs emission levels and biochemical seed quality during storage

The biochemical seed quality attributes *viz.,* the electrical conductivity of seed leachate, lipid peroxidation and lipoxygenase activity was increased and dehydrogenase activity, catalase and peroxidase activity were decreased due to an increase in the release of volatile organic compounds during storage.

The electrical conductivity of seed leachate was increased from 0.263 dSm^{-1} at 1st month of ageing to 0.539 dSm^{-1} at 12th month of ageing. The lipid peroxidation was increased from 0.278 OD value in the 1st month of ageing to 0.548 OD value in the 12th month of ageing. Similarly, the lipoxygenase activity was increased from 0.189 OD value at 1st month of storage to 0.512 OD value at 12th month of storage. In contrast, the dehydrogenase activity was decreased from 1.914 OD value at 1st month of ageing to 0.902 OD value at 12th month of ageing and the catalase activity was reduced from 1.092 µmol H_2O_2 min^{-1} g^{-1} protein at 1st month to 0.728 µmol H_2O_2 min^{-1} g^{-1} protein at 12th month of storage. Similarly, the peroxidase activity was also reduced from 0.481 U mg^{-1} protein min^{-1} at 1st month to 0.211 U mg^{-1}protein min^{-1} at 12th month of storage. At the same time, the strength of total volatiles was 12.89% in 1st month and increased to 54.90% in 12th month of storage. The individual strength of volatile groups *viz.,* Alcohols (1.14%), Aldehydes (2.70%), Acids (3.13%), Esters (4.75%), Alkanes (1.17%), Alkenes (0%), Ketones (0%) and Ethers (0%) was higher in 1st month of storage which further increased to 14.28% (Alcohols), 4.02% (Aldehydes) 9.88% (Acids), 10.88% (Esters), 5.43% (Alkanes), 1.04% (Alkenes), 5.27% (Ketones) and 4.10% (Ether) in 12th month of storage (Fig. 2, 3 & 4).

The loss of biochemical seed quality characters might be due to the increased level of VOCs, which might have been produced by the cell membrane's catabolic event and also due to the toxic effect of free radicals. Volatiles emitted from stored seeds were found to reduce the biochemical quality parameters in pine (Tammela *et al.,* 2003) and cabbage (Bicanic *et al.,* 2003). According to the report of Min *et al.* (2017), Oenel *et al.* (2017) and Bhattacharjee (2019), the release of aldehydes, ketones, alkanes, carboxylic acids and other polymerization products produced due to lipid peroxidation process have been found to diffuse and easily penetrate in to the biological membrane in seed and also affect other cellular and extracellular matrix components of the cell that leads to reduce the biochemical seed quality.

Conclusion

The study demonstrated the influence of volatile organic compounds emission level on physiological and biochemical properties of rice var. Co 51 seeds during storage. There was a significant decrease in physiological and biochemical quality attributes due to increase in the strength of volatiles released during ageing. When the release of total volatile strength reached more than 40%, there was a significant (P=0.05) reduction in physiological attributes such as germination, root and shoot length, dry matter production and vigour index. With respect to biochemical properties, a significant increase in electrical conductivity of seed leachate, lipid peroxidation and lipoxygenase activity, and a decrease in dehydrogenase activity, catalase activity and peroxidase activity was observed. However, the highest reduction in all these properties was recorded when the total volatile strength reached 54.90%. Finally, the study concluded that eight different categories of volatiles were profiled in rice seeds during storage. Among all the volatile organic compounds, 1-hexanol, 1-butanol, ethanol, hexanal, acetic acid, hexanoic acid and methyl ester were the most closely associated volatiles with seed deterioration. It indicated that these components could be considered as the signature components for assessing the seed quality in rice during storage.

Conflict of interest

The authors declare that they have no conflict of interest.

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